SUPPLEMENTAL MATERIAL:

SUPPLEMENTAL METHODS

Control Cardiac Tissues: In prior studies of human heart failure tissue, control tissue was often procured from donor hearts that were not used for transplantation. These hearts had undergone a preservation process that included the use of cardioplegic solution and an indeterminate amount of time on ice.[1, 2] Our control samples were the non-failing left ventricular (NFLV) tissue directly procured from explanted beating hearts from patients who had undergone heart-lung transplantation for right ventricle failure.

Protein expression: Heart tissue was homogenized in cell extraction buffer (Invitrogen) and cell lysates 50-100 ug were loaded on SDS-PAGE gels for separation. Proteins were transferred to a nitrocellulose membrane using standard Western blot techniques. Antibodies to ATP5a1 (complex V), UQCRC1 (complex III), SDHB (complex II), and Cox II (complex IV) were purchased from Mitosciences (Abcam), P53 antibody was purchased from Cell Signaling Technology and used at a concentration of 1:1000 overnight at 4^0C. HRP-conjugated anti-mouse secondary antibodies (Cell Signaling Technology) were used at 1:5000 concentration for 1 hour at room temperature. Proteins were visualized with an enhanced chemiluminescent substrate (Thermoscientific) and bands were quantified with Scion Image and Image J programs. Whole lysate proteins were normalized to GAPDH and mitochondrial proteins were normalized to VDAC1 (porin).

Metabolomics: The procured human LV tissue was trimmed to remove any fat, fibrosis and reduce its size to about 50 mg. An accurate weight of the tissue was noted before metabolic extractions, and all final readings for metabolites were normalized to this starting weight of the tissue. Fifteen amino acids and urea cycle intermediates, 66 acyl-carnitine derivatives, and 7

organic acids (TCA cycle intermediates and related analytes) were measured in human heart samples. Amino acids and acylcarnitine species were measured using flow injection tandem mass spectrometry (MS/MS) and sample preparation methods described previously.[3, 4] Briefly, samples were equilibrated with a cocktail of internal standards and de-proteinated by precipitation with methanol. Aliquoted supernatants were dried, and then esterified with hot, acidic methanol (acylcarnitines) or *n*-butanol (amino acids). The data were acquired using a Waters Acquity UPLC system equipped with a TQD (triple quadrupole detector) and a data system controlled by MassLynx 4.1 operating system (Waters, Milford, MA).[3, 5] Organic acids were quantified using a previously described method that utilizes Trace GC Ultra coupled to a Trace DSQ MS operating under Excalibur 1.4 (Thermo Fisher Scientific, Austin, TX).[4]

All MS analyses employed stable-isotope-dilution. The standards serve both to help identify each of the analyte peaks and provide the reference for quantifying their levels. Stable-isotope internal standards were obtained from Isotec (St. Louis, MO), Cambridge Isotope Laboratories (Andover, MA), and CDN Isotopes (Pointe-Claire, Quebec, CN) to samples, as follows: Acylcarnitine assays--D_3-acetyl, D_3-propionyl, D_3-butyryl, D_9-isovaleryl, D_3-octanoyl, and D_3-palmitoyl carnitines; Amino acid assays--$^{15}N_1$,$^{13}C_1$-glycine, D_4-alanine, D_8-valine, D_7-proline, D_3-serine, D_3-leucine, D_3-methionine, D_5-phenylalanine, D_4-tyrosine, D_3-aspartate, D_3-glutamate, D_2-ornithine, D_2-citrulline, and D_5-arginine; Organic acid assays--D_3-lactate, D_3-pyruvate, $^{13}C_4$-succinate, D_2-fumarate, D_4-glutarate, $^{13}C_1$-malate, $^{13}C_4$-*alpha*-ketoglutarate, and D_3-citrate. In addition to mass, analytes are identified on the basis of the particular MS/MS transitions that we monitor for each class of metabolites. For example, all acylcarnitine methyl esters produce a fragment m/z 99. We make the assumption that all even mass precursors ions of

m/z 99 are acylcarnitines to which we assign plausible molecular structures. Undetectable levels of certain acylcarnitine species were reported as 0.00 nmol/mg tissue.

SUPPLEMENTAL TABLE 1:

Medications: no. (%)	**NFLV n=6**	**HF n=6**	**PostLVAD n=6**
ACEI or ARB's	0 (0)	3 (50)	3 (50)
BB	2 (33)	6 (100)	6 (100)
Diuretics	0 (0)	5 (83)	2 (33)
Aldosterone antagonist	1 (17)	2 (33)	2 (33)
Digoxin	1 (17)	2 (33)	4 (67)
warfarin	3 (50)	4 (67)	6 (100)
Statins	0 (0)	3 (50)	4 (67)
Insulin	0 (0)	2 (33)	3 (50)
Prednisone	1 (17)	0 (0)	0 (0)
ETR antagonist	1 (17)	0 (0)	0 (0)
sildenafil	1 (17)	0 (0)	0 (0)

ETR: endothelin receptor antagonist, ACEI: angiotensin converting enzyme, ARB: angiotensin receptor blocker, BB: beta blocker.

SUPPLEMENTAL TABLE 2:

Table of Demographics (GROUP 2)	
Number	n=6
Age	63 ± 9.4
Sex	
Male	5 (83%)
Female	1 (17%)
Race	
Caucasian	3 (50%)
African American	2 (33%)
Hispanic	1 (17%)
Etiology	
Ischemic	6 (100%)
Non-ischemic	0 (0%)
Device	
Novacor	3 (50%)
DeBakey	3 (50%)
Median #days on support	54.5

SUPPLEMENTAL FIGURES

Fig. S1. Metabolic profiling: (A) Acyl carnitine species, (B) ratios of C4/C18:1 and C4/C18, and (C) amino acid species not included in Figure 2 are shown here. (Mean±SEM; multiple testing analyses using Benjamini and Hochberg adjustment. *P<0.05 versus HF and †P<0.05 versus NFLV).

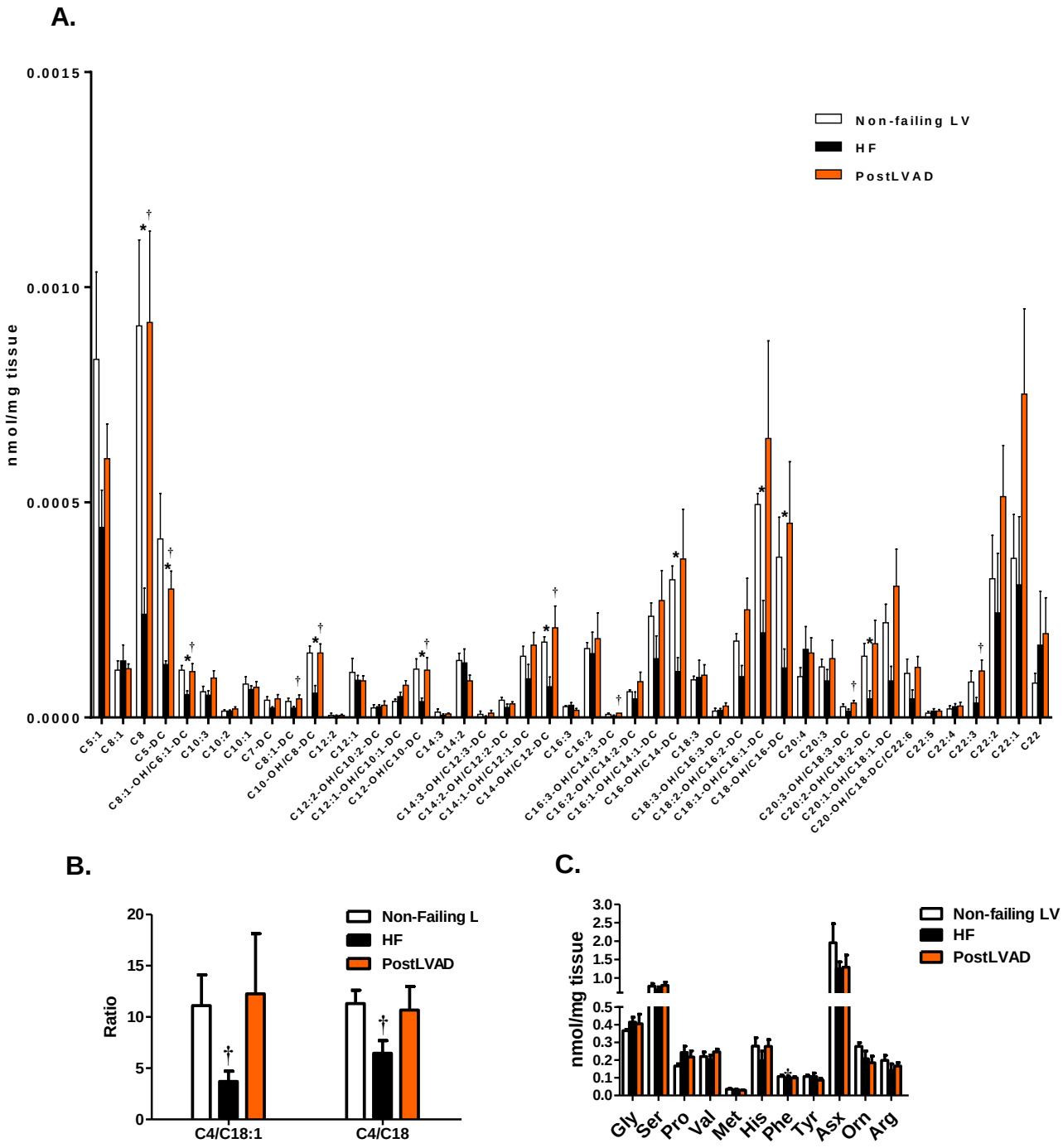


Fig. S2. Effect of diabetes on metabolomic profile of HF patients. Levels of organic acids and acylcarnitine species in diabetic and non-diabetic patients with HF or post-LVAD have been shown. N=3 diabetic, N=3 non-diabetic.

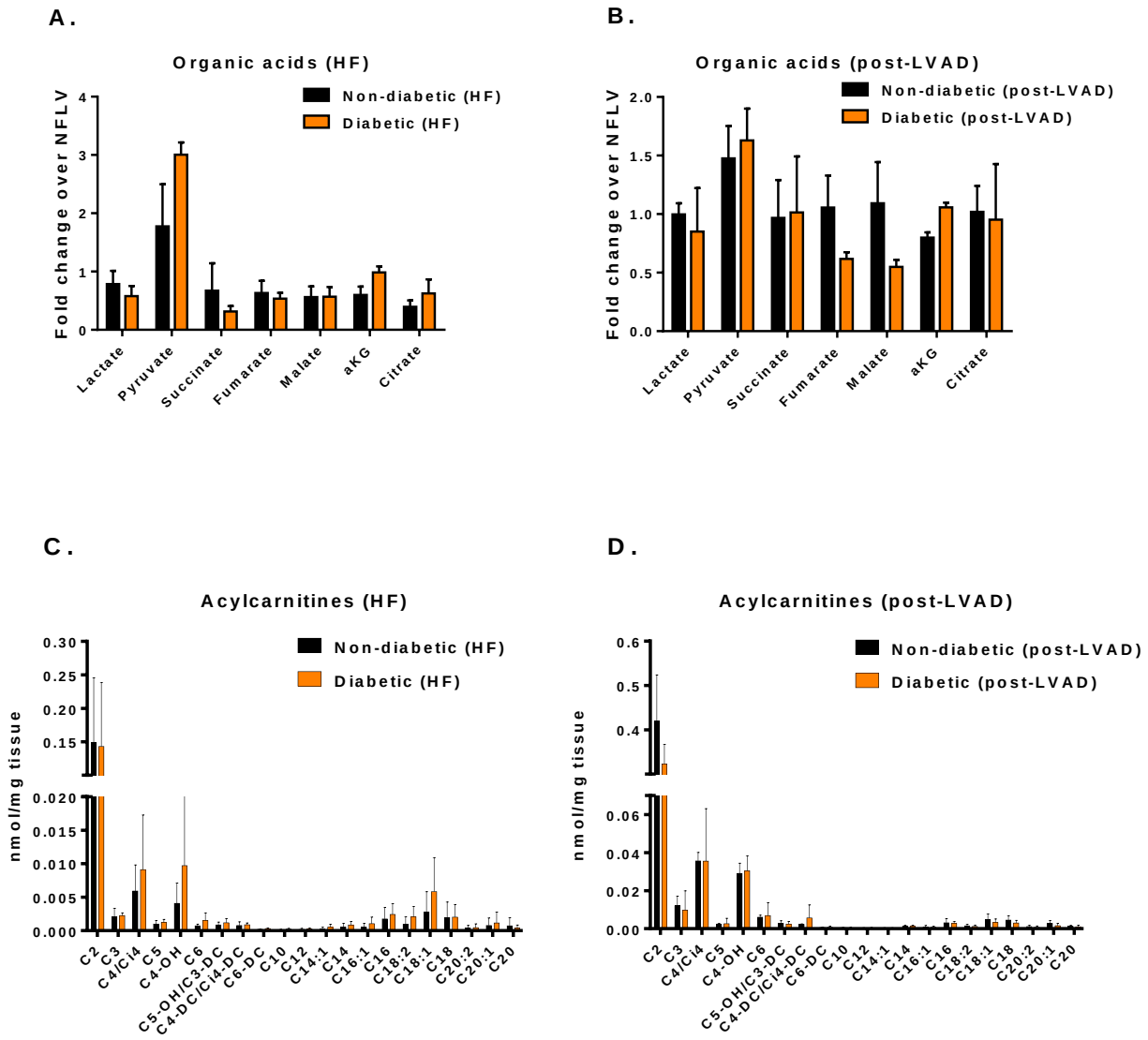


Fig. S3. Gene expression: Expression for candidate genes not included in Fig 3. are shown here. (Mean±SEM; *P<0.05 versus HF by paired Wilcoxon signed rank test; †P<0.05, ††P<0.01 versus NFLV by Mann Whitney test).

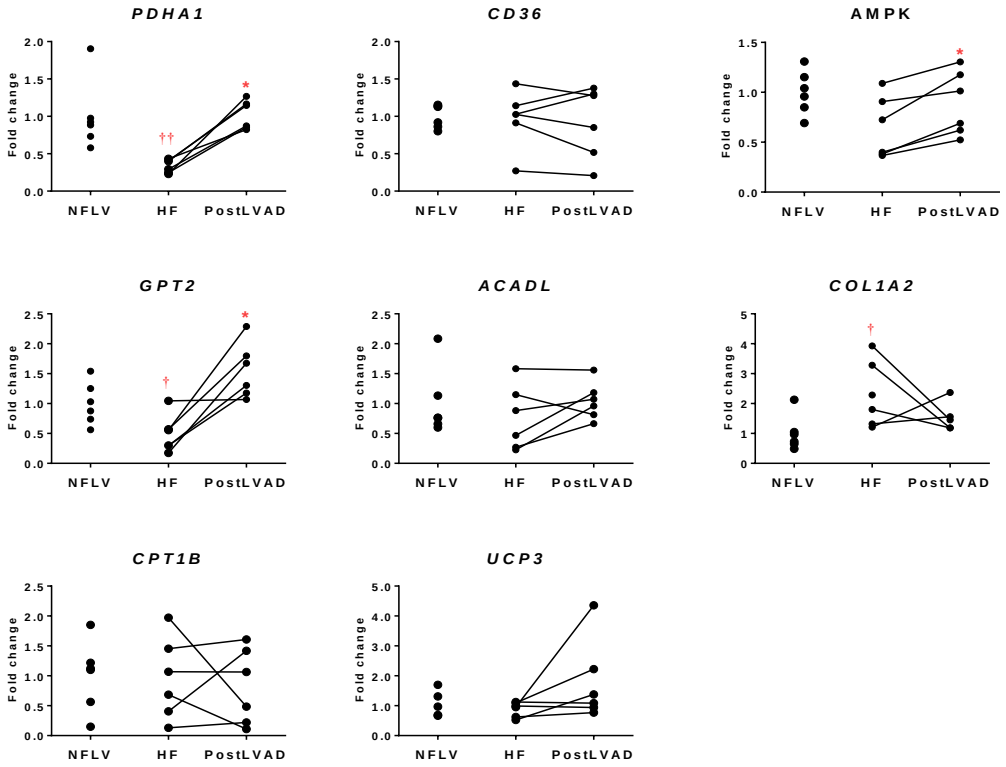
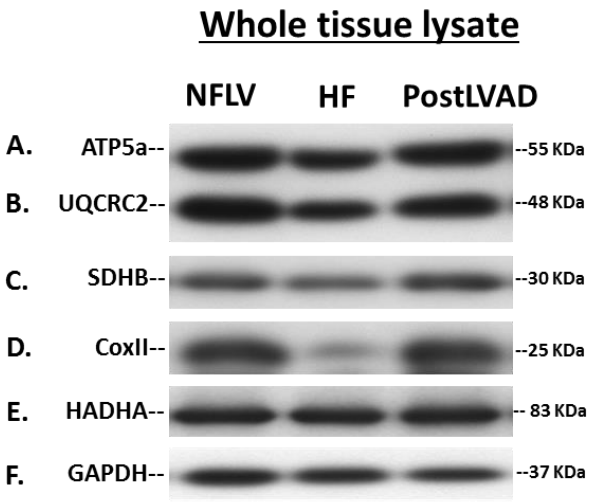


Fig. S4. Protein expression. Western blots showing protein expression in whole tissue lysates for mitochondrial complex proteins (ATP5A, UQCRC2, SDHB, COXII), HADHA and loading control GAPDH.



LITERATURE CITED FOR SUPPLEMENTARY APPENDIX

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